Enzymatically-Responsive Delivery of Pro-Angiogenic Peptides from Poly(ethylene glycol) Hydrogels

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Statement of Purpose: The inability to sufficiently vascularize tissue engineered constructs drastically limits their size, complexity, and potential for clinical translation. Therefore, tissue engineering approaches, as well as a plethora of ischemic tissue disorders, may benefit from pro-angiogenic therapies. Compared to large angiogenic proteins, peptides that mimic growth factor bioactivity allow for delivery of higher concentrations of angiogenic factors and overcome growth factor stability issues. As tight temporal control over growth factor availability is critical for the formation of mature, stable vessels, we aimed to develop a method for sustained, stimuli-responsive release of pro-angiogenic peptides. Thus, poly(ethylene glycol) (PEG) hydrogels were designed to provide sustained, stimuli-responsive release of pro-angiogenic peptides. Peptides were incorporated using tethers responsive to matrix metalloproteinases (MMPs) that are expressed at increased levels in hypoxic and ischemic tissues [1] (Fig. 1).

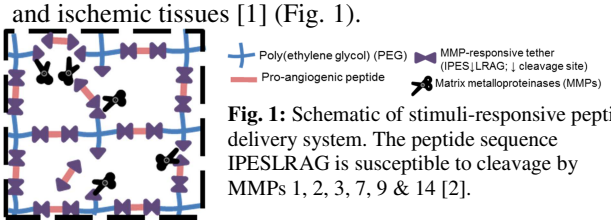

Fig. 1: Schematic of stimuli-responsive peptide delivery system. The peptide sequence IPESLRAG is susceptible to cleavage by MMPs 1, 2, 3, 7, 9 & 14 [2].

Methods: Peptides were synthesized using solid phase peptide synthesis (Liberty1, CEM) [3]. *In vitro* screening of nine peptides from literature identified three that retained native pro-angiogenic peptide ("N") bioactivity in the form released upon liberation from hydrogel networks (with residual peptide from MMP degradable linkers, e.g., LRAG-pro-angiogenic peptide-IPES; "2T"): Qk (a Vascular Endothelial Growth Factor (VEGF) mimic), and $SPARC_{113}$ and $SPARC_{118}$ (derived from the Secreted Protein Acidic and Rich in Cysteine). The pro-angiogenic efficacy of these peptides and the impact of the "2T" forms were assessed using the human umbilical vein endothelial cell (HUVEC) proliferation and tube formation assays [4] (positive control VEGF, negative controls scrambled peptide and the angiogenic inhibitor sulforaphane). Norbornene-functionalized PEG was synthesized and reacted with MMP-releasable pro-angiogenic peptides with cysteine-based thiol functionalities to form hydrogels via thiol-ene reactions [5]. Enzymatically-responsive hydrogel degradation and peptide release upon incubation with MMP-2 was analyzed via hydrogel mass loss and high performance liquid chromatography (HPLC).

Results: Qk, $SPARC_{113}$, and $SPARC_{118}$ resulted in significant increases in HUVEC proliferation (Fig. 2A) and tube formation (Fig. 2B) in "N" and "2T" forms with at least one concentration tested. Interestingly, the "2T" form affected peptide bioactivity differently. Qk in the "N" form induced proliferation at 1 and 100 nM treatment concentrations, while the "2T" form only had proliferative effects at 100 nM. However, tube formation was promoted equivalently in the "N" and "2T" forms at a range of concentrations tested (10 nM to 10 µM;"N" vs. "2T" n.s. by two-way ANOVA).

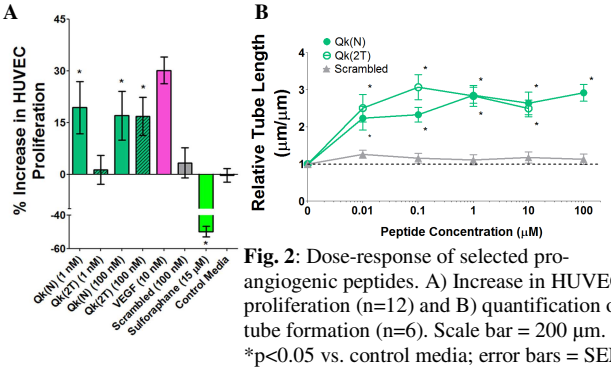

Fig. 2: Dose-response of selected pro-angiogenic peptides. A) Increase in HUVEC proliferation (n=12) and B) quantification of tube formation (n=6). Scale bar = 200 µm. *p<0.05 vs. control media; error bars = SEM.

Enzymatically-responsive release of $SPARC_{118}$ was achieved by flanking the peptide with substrates for MMP-mediated cleavage. Hydrogels were stable in buffer solution alone, and controlled, enzymatically-responsive hydrogel degradation and peptide release from hydrogels occurred over 30 hours upon treatment with MMP 2 (Fig 3A & B), compared to encapsulated peptide which rapidly released in buffer alone in ~6 hours (Fig 3C).

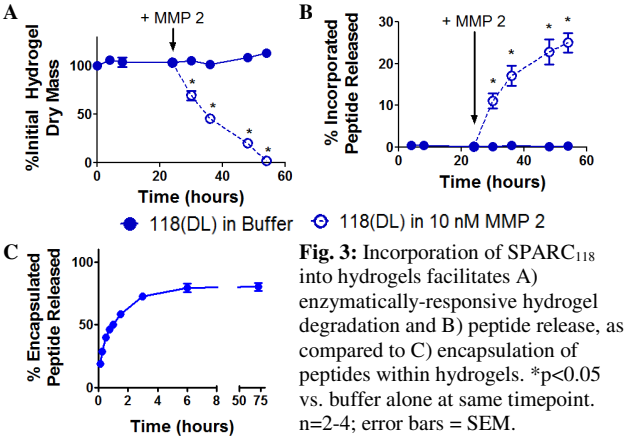

Fig. 3: Incorporation of $SPARC_{118}$ into hydrogels facilitates A) enzymatically-responsive hydrogel degradation and B) peptide release, as compared to C) encapsulation of peptides within hydrogels. *p<0.05 vs. buffer alone at same timepoint. n=2-4; error bars = SEM.

Conclusions and Future Directions: Pro-angiogenic effects of Qk, $SPARC_{113}$ and $SPARC_{118}$ in their "N" and "2T" forms was assessed, clarifying the impact of the "2T" form on peptide bioactivity, with results varying based on the peptide and assessment method. $SPARC_{118}$ was incorporated into PEG hydrogels via the degradable linker IPESLRAG, achieving controlled, enzyme-responsive peptide release. Our current research is focused on demonstrating *in vitro* pro-angiogenic effects of hydrogel-released peptides, as well as *in vivo* angiogenesis within subcutaneous implants and the promotion of angiogenesis and restoration of blood flow within hindlimb ischemia models.

References: [1] Muhs, B.E. *et al. J Surg Res*. **111**(1) 2003. [2] Hubbell, J.A. *et al. Biomaterials*. **31**(30) 2010. [3] Van Hove, A.H. *et al. J Vis Exp*. (80) 2013. [4] Auerbach, R. *et al. Clin Chem*. **49**(1) 2003. [5] Fairbanks, B.D. *et al. Adv Mater*. **21**(48) 2009.